IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Yoshiyuki Nagai et al.
Serial No.: 09/132,521
Filed: 08/11/98
Art Unit: 1633
Examiner: Carrie Stroup
Title: RECOMBINANT SENDAI VIRUS VECTOR EXPRESSING CHEMOKINE

Commissioner of Patents and Trademarks
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, Yoshiyuki Nagai, declare that:

1. I am a co-inventor of the subject matter described and claimed in the present application;

2. I am a co-author of the subject matter disclosed in Moriya et al., "Large quantity production with extreme convenience of human SDF-1α and SDF-1β by a Sendai virus vector," FEBS Letters 425 (1998) 105-111 ("the FEBS Letters paper"). The FEBS Letters paper also names as co-authors, Chikaya Moriya, Tatsuo Shioda, Kei Tashiro, Takashi Nagasawa, Masaya Ikegawa, Yukano Ohnishi, Atsushi Kato, Huiling Hu, Xiaomi Xin, Mohammad K. Hasan, Midori Maekawa, Yutaka Takada, Yuko Sakai, and Tasuku Honjo.

3. So far as I am aware the issue of FEBS Letters containing the FEBS Letters paper was not mailed to the public as early as August 11, 1997, i.e., more than one year before the filing date of the present application.

4. Tatsuo Shioda, Chikaya Moriya, and I are inventors of the subject matter claimed in this application, and together, we are responsible for the inventive input of the subject matter disclosed in the FEBS Letters paper.

5. At the time of the invention, I worked as the director in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan.

6. At the time of the invention, Kei Tashiro worked as the associate professor in the Center for Molecular Biology and Genetics, Kyoto University, Kyoto, Japan. Kei Tashiro provided us the SDF-1 antibody.

7. At the time of the invention, Takashi Nagasawa worked as the chief investigator in the Department of Immunology, Research Institute, Osaka Medical

Center for Maternal and Child Health, Osaka, Japan. Takashi Nagasawa provided us mouse SDF-1 DNA.

8. At the time of the invention, Masaya Ikegawa worked as a research associate in the Department of Social Medicine, Kyoto University, Kyoto, Japan. Masaya Ikegawa provided us SDF-1 antibody.

9. At the time of the invention, Yukano Ohnishi worked as a post doctoral fellow in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Yukano Ohnishi carried out the purification of SDF-1 under my direction and supervision.

10. At the time of the invention, Atsushi Kato worked as a research associate in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Atsushi Kato carried out the establishment of Sendai virus rescue system from cDNA under my direction and supervision.

11. At the time of the invention, Huiling Hu worked as a graduate student in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Huiling Hu carried out the Ca^{2+} influx assay under my direction and supervision.

12. At the time of the invention, Xiaomi Xin worked as a graduate student in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Xiaomi Xin carried out the isolation of HIV-1 from patients under my direction and supervision.

13. At the time of the invention, Mohammad K. Hasan worked as a graduate student in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Mohammad K. Hasan carried out the construction of the background plasmid of Sendai virus cDNA to which SDF-1 cDNAs were to be inserted under my direction and supervision.

14. At the time of the invention, Midori Maekawa worked as a post doctoral fellow in the Department of Pharmacology, Kyoto University, Kyoto, Japan. Midori Maekawa provided us HIV-1 LTR-luciferase plasmid, pHIV-1 LTR/L-A-5'438.

15. At the time of the invention, Yutaka Takebe worked as the laboratory chief in the Department of Molecular Virology and Epidemiology, AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan. Yutaka Takebe provided us HIV-1 LTR-luciferase plasmid, pHIV-1 LTR/L-A-5'438.

16. At the time of the invention, Yuko Sakai worked as a technical assistant in the Department of Viral Infection, Institute of Medical Science, University of Tokyo, Tokyo, Japan. Yuko Sakai carried out the establishment of Sendai virus

rescue system from cDNA under my direction and supervision.

17. At the time of the invention, Tasuku Honjo worked as the director in the Department of Medical Chemistry, Kyoto University, Kyoto, Japan. Tasuku Honjo provided us SDF-1 antibody.

18. I further declare that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

March 8, 2001

Date

Yoshiyuki Nagai

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1. I am a research scientist at DNAVEC Research Inc., the assignee of the above-referenced application. I have technical experience in the research fields of Molecular Biology, Virology, Biochemistry, Protein Chemistry etc. I am also an author of over nine publications. with over two years experience in research concerning the Sendai virus vector. A copy of my curriculum vitae is attached as Appendix A.

2. I am making this Declaration to provide relevant facts in support of the patentability of the subject matter claimed in the patent application

3. I have read and understood the outstanding Office Action mailed on September 12, 2000.

4. I understand that the Examiner contends the inventions of claims 1-8, 11, 12, 14, and 15 to be unpatentable over Yu et al. in view of Bluel et al.

5. To overcome this obviousness rejection and to demonstrate that generally, protein expression by the Sendai virus cannot be foreseen, I submit the experiment data described below.

6. In this experiment, the production efficiency of nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) when using a recombinant Sendai virus vector was determined as follows.

7. The Sendai virus vectors containing a mouse nerve growth factor (NGF) gene (SeV/NGF) or a mouse glial cell line-derived neurotrophic factor (GDNF) gene were prepared by a well-known method using the following primers.

Mouse NGF
N-terminal primer:
5'- ACTTGCGGCCGCCAAAGTTCAGTAATGTCCATGTTGTTCTACACTCTG –3'
C-terminal primer:
5'- ACTTGCGGCCGCGATGAACTTTCACCCTAAGTTTTTCTTACTACGGTCAGCCTCT
TCTTGTAGCCTTCCTGC –3'

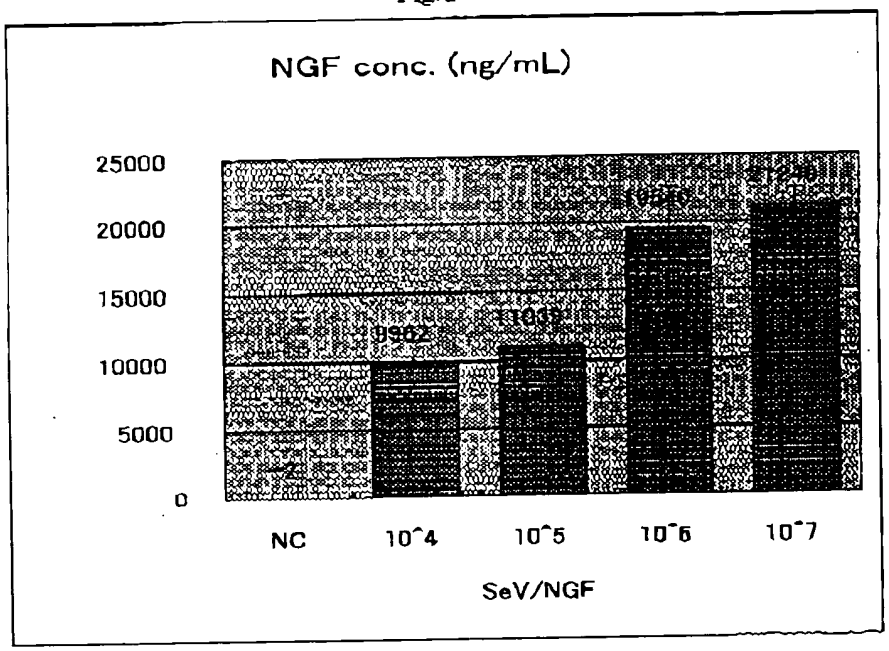
Mouse GDNF
N-terminal primer:
5'- AGTTGCGGCCGCCAAAGTTCAATGAAGTTATGGGATGTCGTGG –3'

C-terminal primer:

5'- ACGTGCGCCGCGATGAACTTTCACCCTAAGTTTTTCTTACTACGGGGGTCAGATA CATCCACACCGTTTAGCGG –3'

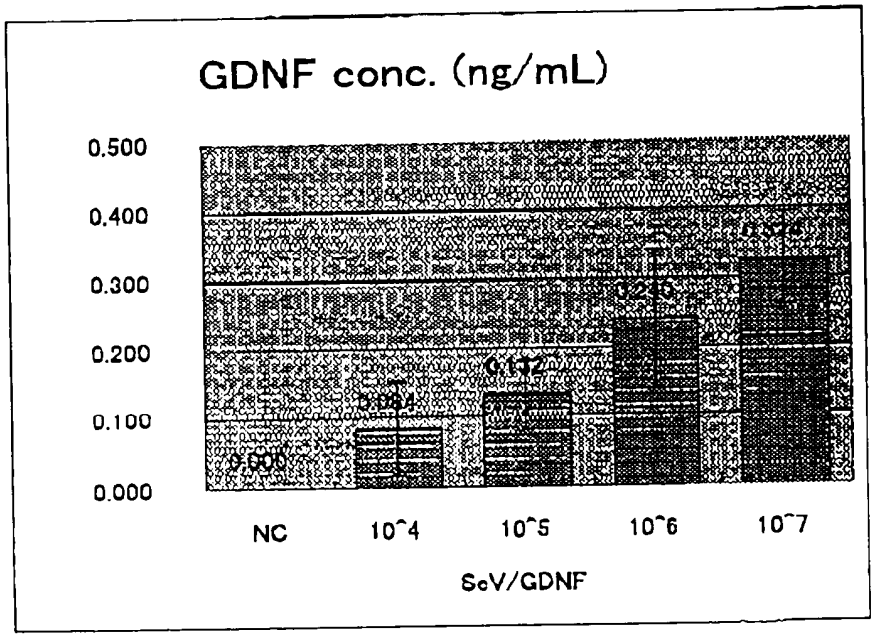
8. Figure 1 shows the production efficiency NGF 72 hrs after infection.

Fig.1



9. Figure 2 shows the production efficiency GDNF 72 hrs after infection.

Fig. 2



10. The above data are summarized in Table 1 below.

Table 1

Infected viral conc. (pfu/ml)	NC	10^4	10^5	10^6	10^7
GDNF conc. (ng/ml)	0.00	0.084	0.132	0.240	0.324
NGF conc. (ng/ml)	-2	9962	11039	19540	21249

NGF was produced 60000 to 80000 better than GDNF. There was no difference in the proliferation of recombinant viruses after infection. Hemagglutination test (HA), a viral assay, revealed an equivalent titer (2^{11} dilution), indicating that a foreign gene does not affect the replication of the Sendai virus. Thus, the above data show that although the exogenous gene has no influence on the replication of the Sendai virus itself, it influences the production efficiency of the proteins when using a Sendai virus vector, to an extent that the effect of the vector-protein combination cannot be predicted beforehand.

11. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C.§ 1001 and that such willful false statements may jeopardize the validity of any patent issuing from the present patent application.

March 9, 2001
Date

Makoto Inoue
Makoto Inoue, Ph. D.